## Scientific Abstract

**A Phase II Trial of Vaccination with Autologous, Lethally Irradiated Melanoma Cells Engineered by Adenoviral Mediated Gene Transfer to Secrete Granulocyte-Macrophage Colony Stimulating Factor in Stage III and IV Metastatic Melanoma Patients**

This Phase II trial for patients with metastatic melanoma will investigate the use as therapeutic vaccines of autologous, irradiated melanoma cells engineered by adenoviral mediated gene transfer to secrete human granulocyte-macrophage colony stimulating factor (GM-CSF). A total of up to 70 evaluable patients will be immunized subcutaneously and intradermally with either $1x10^7$, $4x10^6$, $1x10^6$ or 1/6 of the total yield of engineered autologous melanoma cells weekly times three and then every two weeks until the supply is exhausted.

Our previous Phase I trial of vaccination with lethally irradiated, autologous melanoma cells engineered by retroviral mediated gene transfer to secrete GM-CSF established the safety and biologic activity of this immunization scheme in metastatic melanoma patients. However, the complexity of vaccine manufacture presented a significant impediment to more detailed clinical investigation. We thus conducted a second Phase I trial employing adenoviral mediated gene transfer; this simplified method of vaccine production proved feasible, safe and immunogenic. We now propose a Phase II study aimed at extending these clinical findings in two important ways.

First, we will evaluate the feasibility, safety, and toxicity of this vaccination strategy in stage III melanoma patients. Subjects may be enrolled if they manifest gross lymphadenopathy and either refuse, fail, or are ineligible for adjuvant therapy with high-dose α-interferon. These investigations will yield new information regarding the doses of vaccines that can be manufactured in the context of regional disease as compared to the previous studies in disseminated disease. Moreover, the generation of host responses in stage III patients (who also likely received less prior therapy than stage IV patients) may differ from the earlier studies; thus, we will analyze both toxicity and immunity in this patient cohort. If this trial reveals significant biologic activity without substantive toxicity, then these results would provide a foundation for undertaking a subsequent Phase III study testing the therapeutic efficacy of vaccination in the adjuvant setting.

Second, we will more thoroughly evaluate the biologic activity of this vaccination scheme in stage IV melanoma patients. The prolonged survival (at least 44 months) of 10 of 35 subjects in the previous Phase I trial is intriguing, in view of the mean survival of only 6-9 months for patients with metastatic disease. Indeed, survival may prove to be a more informative clinical endpoint than tumor regression for this vaccination scheme. Pathologic analysis of distant metastases following immunization revealed

extensive tumor necrosis (at least 90%) associated with inflammatory infiltrates and fibrosis in nearly two-thirds of the patients examined. However, these reactions were only rarely associated with tumor regression, as determined by standard clinical criteria; instead the masses underwent a marked alteration in cellular composition. Thus, we propose to determine in a larger cohort of stage IV patients the two-year survival associated with vaccination. Lastly, blood and tumor samples obtained during the course of immunization function as critical reagents for detailed analysis of vaccine-induced immune responses. Indeed, our laboratory investigations already have identified several novel targets of immune-mediated tumor destruction; these include ATP6S1, melanoma-inhibitor of apoptosis protein (ML-IAP), opioid growth factor receptor (OGFr), CML28, and CML66.

The overall goals of the proposed phase I study are:

1.1 To determine the doses of lethally irradiated, autologous melanoma cells engineered by adenoviral mediated gene transfer to secrete GM-CSF that can be manufactured in stage III melanoma patients.

1.1.1 To determine the safety and biologic activity of vaccination with lethally irradiated, autologous melanoma cells engineered by adenoviral mediated gene transfer to secrete GM-CSF in stage III melanoma patients

1.2 To determine the two-year survival of stage IV melanoma patients vaccinated with lethally irradiated, autologous melanoma cells engineered by adenoviral mediated gene transfer to secrete GM-CSF.

1.2.1 To determine more fully the safety and biologic activity of vaccination with lethally irradiated, autologous melanoma cells engineered by adenoviral mediated gene transfer to secrete GM-CSF in stage IV melanoma patients